2010. The Journal of Arachnology 38:150–152

SHORT COMMUNICATION

Nephila clavipes females have accelerating dietary requirements

Linden Higgins and **Charles Goodnight**: Department of Biology, University of Vermont, 109 Carrigan Drive, Burlington, Vermont 05405, USA. E-mail: Linden.Higgins@uvm.edu

Abstract. *Nephila* spiders are famous for extreme sexual size dimorphism, with females an order of magnitude larger than males. The proximal developmental mechanism for the sexual size dimorphism is extended development in females: they have many more juvenile instars than males. During an experimental rearing of *Nephila clavipes* (Linnaeus 1767) from two populations, we discovered that females cannot reach sexual maturity on diets that are qualitatively and quantitatively sufficient for male maturation. Here we describe the dietary regimes that produced sexually mature females and the life history implications of these requirements.

Keywords: Female gigantism, sexual size dimorphism, SSD, Araneae, Nephilidae

Spiders in the family Nephilidae are famous for extreme sexual size dimorphism, with males of many species an order of magnitude smaller than the females (Vollrath 1980; Christensen & Goist 1979; Hormiga et al. 2000; reviewed in Kuntner & Coddington 2009). This sexual size dimorphism originates developmentally through delayed maturation in females: they pass through several additional instars, while males mature between the fifth and eighth instar after emergence (pers. obs.; Hormiga et al. 2000). In order to better determine the developmental differences underlying extreme sexual size dimorphism, we developed a protocol for rearing female *Nephila clavipes* (Linnaeus 1767) (Araneae: Nephilidae) in the laboratory. Here, we describe the developmental trajectories, food requirements, and mortality patterns of females; male data have been presented elsewhere (Higgins & Goodnight unpubl. results). Most striking from these results is that in terms of the mass of food required for development (relative to body mass), females have accelerated dietary requirements after about the seventh instar, coinciding with a previously observed decline in orb-web investment (Higgins 2006) and likely reflecting accelerated growth rates associated with maturing early in strongly seasonal environments.

Seven egg sacs with unhatched eggs were collected in Los Tuxtlas Biological Research Station, Veracruz, Mexico, and shipped to Vermont, USA; all but one of these sacs failed to hatch, apparently due to desiccation of the eggs in transit. Five egg sacs were collected in Brazos Bend State Park, near Houston, Texas, USA (vouchers from these populations have been placed in the National Museum of Natural History, Smithsonian Institution, Washington, D.C.). All of these were hatched and had molted to the first true instar prior to shipping, and arrived alive. Dispersing spiderlings from the six egg sacs (one from Los Tuxtlas, five from Brazos Bend) formed the study population. Because of low survivorship of females during development, data are pooled across all families from the Brazos Bend population. To ease the burden of feeding large numbers of small spiders, we staggered the emergence of Brazos Bend spiderlings by holding egg sacs in cool, short-day conditions in a box lined with damp paper towels in a walk-in refrigerated chamber (4° C, 14:10 h D:L). When starting a new clutch, we hung an egg sac in a large box (31 cm wide x 23.5 cm high x 11 cm deep, Pioneer plastics) on 2.5 cm (= 1 in) chicken wire and placed a tube of high-protein *Drosophila melanogaster* (reared on instant fly food supplemented with high-protein dog chow: Mayntz et al. 2003) in the box, placing the box in warm long-day conditions (25° C, 10:14 h D:L, 75% RH) in a Percival incubator. In addition to releasing *D. melanogaster* into the boxes, we sprayed the spiderlings twice weekly with a dilute pollen solution (0.1 g organic "bee" pollen in 500 ml distilled water). Upon molting to the third instar, we moved spiders into individual boxes and randomly assigned each to a treatment group.

Spiders were fed biweekly most weeks; occasionally a weeks' worth of prey was provided at a single feeding. Initial treatments were: Low = 35% of post-molt body mass/week; Medium = 56%; High = 84%. These diets are in the middle of the range used by Higgins & Rankin (2001), which resulted in normal growth rates without the high mortality associated with overeating. Rather than removing spiders from their webs to weigh them, we estimated the mass of the spiders from the leg 1 tibia + patella length (TPL), abdomen length and abdomen width as in Higgins (1992: mass (mg) = 81 (TPL^3) + 784 (abdomen volume)). For the first four experimental instars, we based diets upon the mean size of the first 4–7 spiders reaching those instars, and spiders were fed *D. melanogaster*. After TPL $\geq$ 0.5 cm, we calculated diets individually for each animal immediately after each molt and fed spiders a mixture of *D. virilis* and *D. melanogaster*. All spiders in the same instar received the same quality of diet. Prey numbers used were calculated based upon the mean mass of each prey type: *D. melanogaster* (mean mass 0.748 mg, SD = 0.110, n = 11), *D. virilis* (mean mass 1.60 mg, SD = 0.239, n = 15). The *D. virilis* were not reared on protein-supplemented diets. At the eighth instar, we added commercially reared, high-protein house flies to the diets (*Musca domestica*, www.SpiderPharm.com; mean mass 11.65 mg, SD = 2.077, n = 10). The shifts in prey type were necessary for logistical reasons: if we had fed only *D. melanogaster* through the entire development, the number of flies provided in later instars would have numbered in the hundreds per week due to the large size of the juvenile females. In subsequent instars, the housefly proportion of the diet by mass increased (*D. virilis*: house flies – eighth instar: 3:7, ninth instar: 2:8, tenth or eleventh instar: 1:9).

As spiders molted to larger sizes, we moved them to accommodate their larger webs. When they molted to the 6th instar (TPL ca 0.3 cm), they were moved to a larger box (22 cm wide x 10 cm high x 10 cm deep), oriented horizontally for smaller spiders (0.3 cm $\leq$ TPL $<$ 0.5 cm) and vertically for larger ones (0.5 cm $\leq$ TPL$<$ 0.7 cm). All but three males reached sexual maturity while in this size range (prior to TPL = 0.6 cm). Juvenile females were moved to the largest box size when they molted to TPL $\geq$ 0.7 cm (31 cm wide x 23.5 cm high x 11 cm deep). To mimic environmental cues in natural populations, all spiders were moved to short-day conditions (11:13 h L:D) in a walk-in chamber 4 mo (= 138 days) after starting the experiment. Most

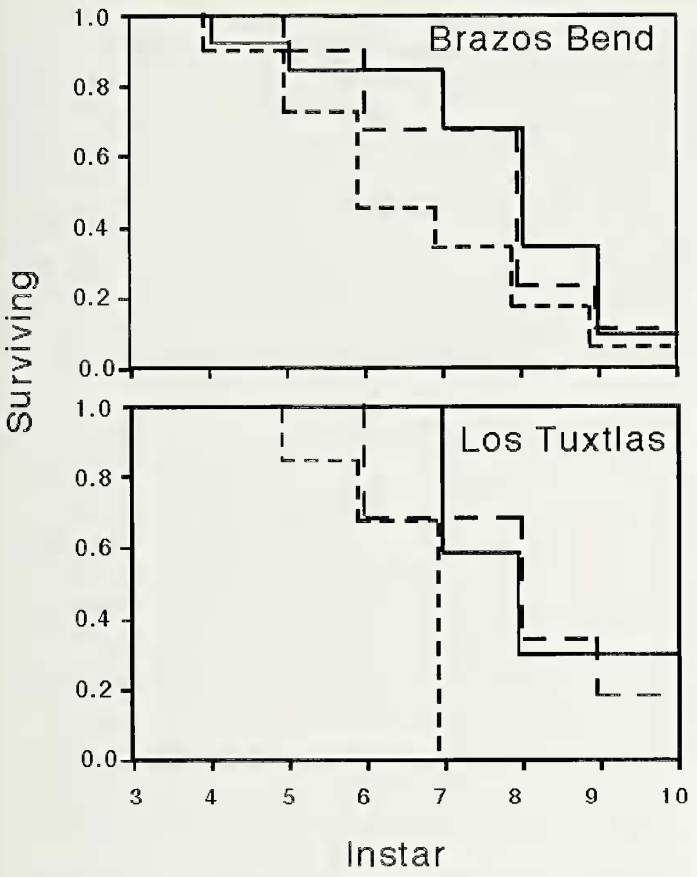

Figure 1.—Survivorship of unsexed juveniles (males and females to the 8th instar) and immature females on the three initial diets (L1: short dash; M1: long dash; H1: solid line), by instar. The survivorship calculations were censored by maturation (i.e., mature animals are removed from the calculation of survival).

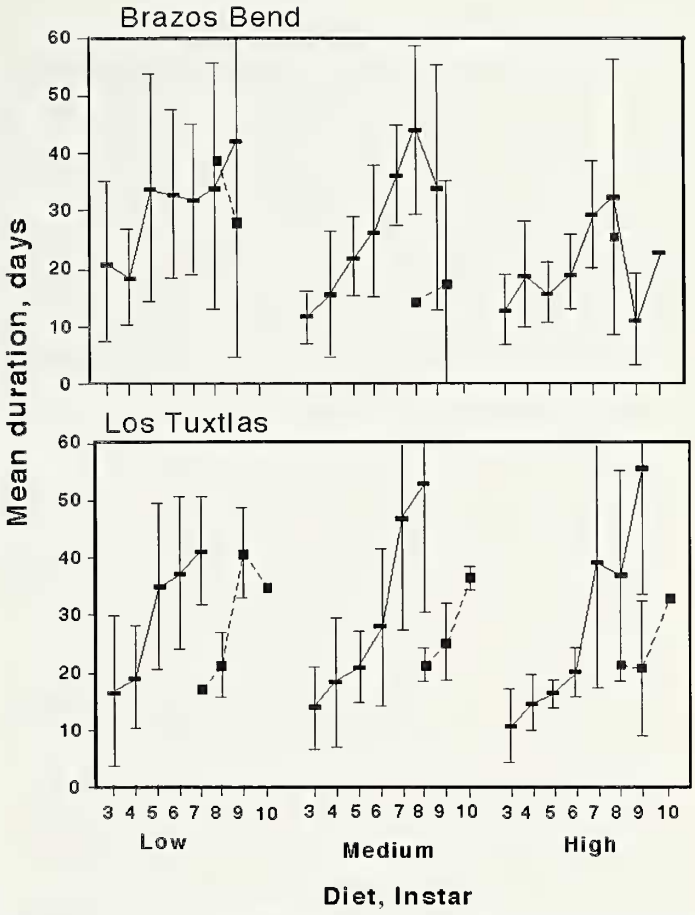

Figure 2.—Average instar duration of juvenile female spiders reared on low, medium, and high diets plotted against instar number. The trajectories on the initial diets (solid lines/bars) bifurcate at the seventh instar when half of the spiders were switched to higher diets (dashed lines/squares). Where no SD is indicated, only a single animal was observed. Note that for Brazos Bend in the H2 diet, only one individual in the eighth instar is represented because spiders molting to the ninth instar were all maturing, and sample sizes were very low.

males were sexually mature at the time of the move. Temperature and humidity in the walk-in chamber were less exactly controlled, but averaged 24° C and 72% RH.

We checked all spiders twice weekly, at which time we recorded and removed all uneaten dead flies, and recorded if the spider had molted. There were no differences in size at the first experimental molt (instar 4) across diets within populations. In addition to measuring the spiders, we retrieved the shed exoskeleton, which serves as a physical record of TPL of the prior instar.

All males from both populations reached maturity by the eighth instar (Brazos Bend: $n = 34$, range = 5–8; Los Tuxtlas $n = 6$, range = 5–7). Prior to the penultimate male instar, males and females cannot be distinguished, and thus the data for instars 3–7 include males and females. Penultimate males are not included in the survivorship analysis, as only 1 penultimate male (from Brazos Bend) died during the experiment.

About the time that males were reaching maturity, we noticed that juvenile female mortality was increasing (Fig. 1). Moreover, even spiders fed the highest diet spent much longer in the seventh instar (mean TPL = 0.58 cm) than the 14-day average for field-observed animals of this size (Higgins 1992; Fig. 2). Average eighth instar duration on the high diet was nearly 30 days for Brazos Bend animals and nearly 40 days for Los Tuxtlas animals. Despite being mid-run on the experiment, we decided to increase the diets of a random half of the individuals by 50% on 18 August 2006, when 22 Brazos Bend females and 21 Los Tuxtlas females were still alive. Because of the staggered start dates for different egg sacs and for spiderlings within an egg sac, the age of the spiders at the time of the shift varied (BB: mean age = 116.5 days, SD = 29.1; LT: mean age = 123.3, SD = 16.7). Most of the animals were in the seventh instar (fourth instar of experimental treatment; BB mean = 7.2, SD = 0.91; LT mean = 7.33, SD = 0.86). This resulted in six final diet treatments for females: low 1 (35%) and low 2 (switched to 56%), medium 1 and 2 (56%, 84%) and high 1 and 2 (84%, 126%). It is noteworthy that none of the animals in the eighth instar at the time of the diet shift reached maturity, even if they received the greater amount of food.

Instar duration shortened dramatically in the spiders experiencing the increase in food availability (Fig. 2). After log-transforming the data to normalize distributions, we tested the effect of diets on development by comparing the age and size of spiders entering the ninth instar for each population with separate MANOVA analyses, followed by individual ANOVA tests to determine how size and age were affected by diet (all statistical analyses performed on JMP 7.0.2). The separation of the two populations was necessary because none of the LT spiders on the L1 treatment survived to the ninth instar. For the Brazos Bend spiders, development to the ninth instar was significantly affected by diet ($n = 13$, partial correlation = 0.025; Roy's Maximum Root = 5.75, DFE = 7, $P = 0.0081$). In these spiders, age at the ninth instar was not altered by diet (ANOVA: $F_{(5, 12)} = 1.37$, $P = 0.34$), but spiders on higher diets were significantly larger (ANOVA: $F_{(5, 12)} = 7.55$, $P = 0.01$). The Los Tuxtlas spiders also showed a significant developmental response to diet and the two

developmental parameters were correlated with each other (n = 14, partial correlation = 0.53; Roy's Maximum Root = 5.64, DFE = 9, P = 0.015). The Los Tuxtlas spiders on higher diets showed significantly faster development to the ninth instar (ANOVA: $F_{(4, 13)} = 4.34$, P = 0.032; but no change in size with diet (ANOVA: $F_{(4, 13)} = 1.27$, P = 0.35).

A total of 14 females reached sexual maturity, four from Brazos Bend and ten from Los Tuxtlas. With the apparent difference in developmental response to diet, the data cannot be pooled across these two populations, and only the Los Tuxtlas sample is large enough to consider dietary effects on female size and age at maturation. We tested for an effect of diet by ranking the six diets from lowest to highest (L1, L2, M1, M2, H1, H2). Among these survivors, neither age nor size at maturity was affected by diet; however, no more than three animals survived from any diet group (standard least-squares regression - size: ln (TPL, mm): $F_{(1, 9)} = 0.72$, P = 0.42; age: ln (days since initiation of experiment): $F_{(1, 9)} = 2.49$, P = 0.15).

In light of the problems of synchronization of development in a species with female gigantism, where the gigantism is proximally caused by the addition of juvenile instars (Hormiga et al. 2000), it is perhaps not surprising that female dietary requirements accelerated at an intermediate developmental stage. These results also help to explain prior descriptions of declining relative investment into foraging by these spiders in Mexico (Higgins 2006): since prey capture rates are not tightly linked to orb-web size (Higgins & Buskirk 1992), spiders may be reducing foraging investment in order to shift resources to growth and development. Despite the high mortality of spiders even after the dietary shift, we do not believe that these results imply qualitative nutritional requirements being unmet for the following reasons. First, many kinds of spiders are regularly reared successfully on protein-enhanced fruit flies and house flies (Mayntz et al. 2003; C. Kristensen, Spiderpharm.com, pers. comm.), including *Nephila fenestrata* Thorell 1859 and *N. edulis* (Labillardière 1799) (N. Ruppel pers. comm.; L. Ceballos Meraz pers. com.). Second, few juveniles die before they can be sexed, and penultimate-instar males almost never die. It appears likely that large juvenile females are starving to death due to lack of food, rather than lack of nutrients in the food they are receiving. This sensitivity to food levels may be a price these spiders pay for the reproductive benefits of large female size.

ACKNOWLEDGMENTS

Rearing large numbers of spiders is time-consuming, and these experiments would not have been possible without the aid of volunteer undergraduates Sarah Wanamaker, Cynthia Quell, Rachel Taylor, Donald Kraft, Lauren Gauthier, Ashley Couture, and Ariel GallentBernstein. Collecting in Texas was hosted by Ruth Buskirk, and Juan Nuñez Fárfan and Jesus Vargas supplied the spider egg sacs from Los Tuxtlas. C. Kristensen, N. Ruppel, and L. Ceballos Meraz were generous with their time in discussing spider diets and these results. An anonymous reviewer, Matthias Foellmer, and Jeffrey Shultz provided comments that greatly improved the presentation of these results. This research was supported by a grant to LEH from the National Science Foundation (0233440).

LITERATURE CITED

Christenson, T.E. & K.C. Goist. 1979. Costs and benefits of male-male competition in the orb-weaving spider, *Nephila clavipes*. Behavioral Ecology and Sociobiology 5:87–92.

Higgins, L.E. 1992. Developmental plasticity and fecundity in the orb-weaving spider *Nephila clavipes*. Journal of Arachnology 20:94–106.

Higgins, L.E. 2006. Quantitative shifts in orb investment during development in *Nephila clavipes* (Araneae: Tetragnathidae). Journal of Arachnology 34:374–386.

Higgins, L.E. & R.E. Buskirk. 1992. A trap-building predator exhibits different tactics for different aspects of foraging behavior. Animal Behaviour 44:485–499.

Higgins, L.E. & M.A. Rankin. 2001. Mortality risk of rapid growth in the spider *Nephila clavipes*. Functional Ecology 15:24–28.

Hormiga, G., N. Scharff & J.A. Coddington. 2000. The phylogenetic basis of sexual size dimorphism in orb-weaving spiders (Araneae, Orbiculariae). Systematic Biology 49:435–462.

Kuntner, M. & J.A. Coddington. 2009. Discovery of the largest orbweaving spider species: the evolution of gigantism in *Nephila*. PLoS ONE 4:e7516. 5 pp.

Mayntz, D., S. Toft & F. Vollrath. 2003. Effects of prey quality and availability on the life history of a trap-building predator. Oikos 101:631–638.

Vollrath, F. 1980. Male body size and fitness in the web-building spider *Nephila clavipes*. Zeitschrift für Tierpsychologie 53:61–78.

Manuscript received 30 October 2008, revised 13 November 2009.